(5627*5)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

A. JAMES MIXSON :

SERIAL NO.: 10/018,103 : **ART UNIT: 1632**

FILED: NOVEMBER 5, 2001 : **EXAMINER: Nguyen, Dave Trong**

FOR: HISTIDINE COPOLYMER AND METHODS FOR USING SAME :

Assistant Commissioner for Patents
Washington, DC 20231

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST-CLASS MAIL IN AN ENVELOPE ADDRESSED TO: ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON D.C. 20231
ON THIS 1st DAY OF June 2004.
BY: Barbara J. Mills

Declaration Under 37 C.F.R. § 1.131

Dr. A. James Mixson declares as follows:

1. I am the sole inventor named on the above-referenced patent application.

2. On a date prior to February 19, 1998, the earliest possible effective filing date of USSN 09/251,783 (Pack *et al.*), which was cited by the examiner, the invention of this application was reduced to practice in the United States.

3. To establish completion of the invention of this application, the following attached documents are submitted as evidence:

a) Custom Peptide Order Form (1 page copy of original with date redacted, labeled "Exhibit A")

b) Mass Spectroscopy Data (1 page copy of original with date redacted, labeled "Exhibit B")

c) Notebook Page (1 page copy of original with date redacted, labeled "Exhibit C")

The dates have been redacted from these documents. The original documents have the dates listed thereon.

4. Exhibit A is a copy of a "Custom Peptide Order Form", submitted to the Biopolymer Laboratory of the Department of Microbiology/Immunology at the University of Maryland School of Medicine requesting synthesis of a 12-mer peptide having the amino acid sequence H-H-H-H-H-K-H-H-H-H-H-K (hereafter referred to as the "HK polymer"). This request form was submitted by me prior to February 19, 1998.

5. Prior to February 19, 1998, mass spectroscopy was performed on the 12-mer HK polymer. Exhibit B is a copy of the results of that analysis. The molecular weight as identified by the mass spectroscopy of the HK polymer was 1645.58, which is almost identical to the theoretical molecular of 1645.86.

6. Prior to February 19, 1998, the ability of the HK polymer to carry a luciferase-expressing plasmid (BAP-Luc) into CHO cells was tested. This experiment is described in a copy of a page from my notebook (Exhibit C). As described in Exhibit C, several different amounts of the HK polymer (referred to as "polyhistidine" or "polyhis" in my notebook) were found to carry the DNA into cells. The top table on the page describes the four different treatments tested. The first (labeled "1)") was untreated BAP-Luc, which served as a control. The "x 3" indicates that three wells of a multi-well cell culture plate used this treatment. In the second through fourth treaments, labeled in the notebook as "polyhis 15", "polyhis 20" and "polyhis 30" respectively,15 µl, 20 µl or 30 µl of a 2 µg/µl solution of HK polymer (for a total of 30, 40 or 60 µg of HK polymer, respectively) was mixed with 0.4 µl of a 1.65 µg/µl solution of BAP-Luc DNA (for a total of 0.66 µg) for 40 minutes. The HK polymer:BAP-Luc complex formed was then added to CHO cell cultures for four hours. The complex was then washed from the cells, and growth media (DMEM + 10% serum) was added. After 24 hours the luciferase activity was measured as Relative Light Units (RLU) in the cell lysate with the Luciferase Assay System (Promega) and with a Turner 20/20 luminometer. The lower table on the page provides the results of the luciferase assay. The mean RLU level for the untreated BAP-Luc DNA was below detectable limits (indicated as 0.00 in the table). The mean RLU level for the polyhis 20 complex was 0.375 RLU (the duplicate values were 0.45 and 0.30

RLU). The mean RLU level for the polyhis 30 complex was 0.76 (0.82 and 0.60 RLU). At these concentrations of HK polymer, the RLU measured far exceeded the detectable value and this verifies that HK polymer by itself can carry the plasmid intracellularly. Nonetheless, based on these results, as indicated on the notebook page, I concluded that "polyhis is [sic] poor carrier by itself." This statement was made in reference to transfection efficiency achievable with commercially available delivery systems. The statement, however, does not detract from the more general conclusion demonstrated by the results that copolymers of histidine and lysine can be used to increase the intracellular delivery of DNA.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATE: 5/28/04

A. James Mixson

Dr. A. James Mixson

School of Medicine

Exhibit A

REQ. #675016

UNIVERSITY OF MARYLAND
AT BALTIMORE

The Biopolymer Laboratory

Department of Microbiology/Immunology
University of Maryland School of Medicine

CUSTOM PEPTIDE ORDER FORM

Principal investigator: Jim Mixson Date: [redacted]
Department and School Pathology
Name of requestor (if other than P.I.): Phone: 6-3223
Account to be charged 02-3-90736-3752
Signature of P.I. or other authorized person:
User sample name: Mix H Bio-Lab sample name: Mix H MIXSON1
(Please note-orders can still be faxed to the Biopolymer Lab; however, this signed form must be presented when picking up order.)

Instructions: Please enter the sequence from the amino terminus, completing the sequence at the carboxyl terminus. Use either the three letter or one letter code.

1-10	NH_2	H	H	H	H	H	K	H	H	H	H
11-20		H	K								
21-30											
31-40											
41-50											
51-60											

Synthesis Scale: 25 µmole 50 µmole 100 µmole 250 µmole
Sequence termination: -COOH -$CONH_2$
Maps Resin: yes no
Purification: yes no

Special services: _____

For Core Use Only:

Charges:	25 µmole	50 µmole	100 µmole	250 µmole
Set-up:	$20	$30	$40	$60
Number of couplings: 11	10 ($10/cp)	____ ($15/cp)	____ ($25/cp)	____ ($30/cp
MAPS Resin:	~~$20~~	$50	$80	$120
Purification:	~~$200~~	$200	$250	$300
Total:	130			

Our phone number is 410-706-3339. If submitting your order from off-campus, our Fax number is 410-706-0287.

Exhibit B

Account: 0

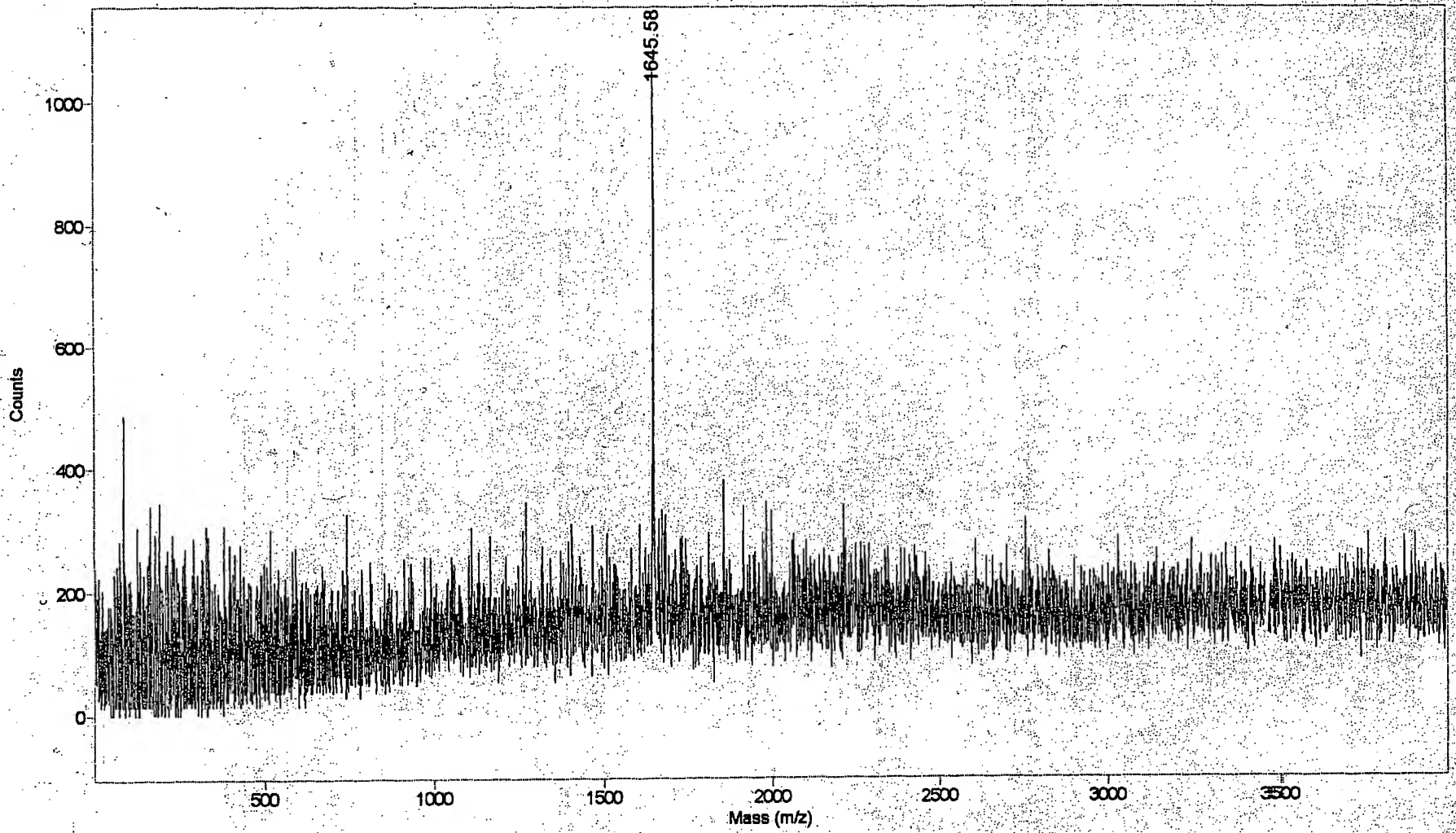
Biopolymer Core Facility

Original Filename: c:\voyager\data\[redacted]\webx_059.ms

This File # 2 = C:\VOYAGER\DATA\MAY97\MIX1.MS

Collected: [redacted]

Sample: 5



BEST AVAILABLE COPY

Comment: NEGATIVE ION

Method: TRAINING

Accelerating Voltage: 30000

Low Mass Gate: OFF

Negative Ions: OFF

Laser: 700

Scans Averaged: 20

Pressure: 5.87e-07

160
10
1600

[redacted]

Exhibit C

Polyplus as a carrier

(HiK-8-1) - titer concentration stock before

BAP-LUC-1.65 mg/ml

polyplus - 2 mg/ml

T.V. 100 ul x 3

DNA

0.4 ul

1) Untreated x 3
2) polyplus 15 ul x 3 - 45
3) 20 ul x 3 - 60
~~25 ul x 3 - 75~~
4) 30 ul x 3 - 90

3 ul in 800 ul of Opti MX 3

Let sit x 40 mins after polymer + DNA are mixed with one another, 2 hrs add 100 ul of Opti M per rxn. Add 540 ul per well after washing cells with PBS

Cats		~~T.V. 100~~ Lysate - 9 ul	Luciferase Buffer 90 ul	RLU
	2			0.00
polyplus	15			0.00
"	15			0.06
"	20			0.08
"	20			0.45
"	30			0.30
"	30			0.82
				0.60

Polyplus is poor carrier by itself